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TRANSLATION

RESULTS OF INVESTIGATIONS OF THE EFFECT OF IONIZATION RADIATION ON THE RETINA AND CERTAIN PHOTOSENSITIVE SYSTEMS

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UNEDITED ROUGH DRAFT TRANSLATION

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RESULTS OF INVESTIGATIONS OF THE EFFECT OF IONIZING RADIATION ON THE RETINA AND CERTAIN PHOTOSENSITIVE SYSTEMS

G. G. Demirchoglyan et al.

The problem of the reactions of the nervous system are of importance when studying the mechanisms of radiation injury to an organism. Is the reaction that portion of an organism which first responds to the effect of ionizing radiations, or are the changes in it secondary owing to physical, chemical, and biochemical changes in the environment? In present-day radiobiology there is hardly any area as little studied as that of the role of the nervous system in radiation damage of an organism. As Academician L. A. Orbeli [1] pointed out, the role of the nervous system is especially apparent in the case of long-term exposure to irradiation in small doses, because under massive effects disorders of vital functions are great and the role of the nervous system can be kept in the background or disguised.

Our laboratory has been investigating the effect of ionizing radiation on the retina, which can be considered as a unique portion of the central nervous system brought out to the periphery. Therefore, any result obtained in the experiments on the retina is of substantial importance for the characteristics of radiosensitivity of the nervous system as well as of interest for evaluating the status of visual reception under these conditions. There is still another characteristic of radiobiological investigations of the retina. The fact is that the results of the electroencephalographic, conditioned-reflex, and other investigations into the effect of ionizing radiation apparently do not characterize the limit of radiosensitivity of the nervous system, since the formations investigated in the aforementioned experiments have evident potentialities for compensating the radiation changes. The retina of an isolated eye, used in certain of our experiments, is extremely suitable from this point of view for solving the problem stated, because it has minimum capacities for compensation of the changes that occurs.

We used different methods to work out the problem of the effect of ionizing radiation on the retina: electroretinography (ERG) (i.e., the recording of the electrical potentials of the retina) with the use of a contact eye lens-electrode, microelectrode techniques for recording the intraretinal potentials, amperemetric determination of the content of the sulfhydryl group in the retina and a measurement of the absorption spectra of the visual pigment, rhodopsin, extracted from the retina, and morphologic and certain histochemical analyses. To ascertain the radiosensitivity of various photosensitive systems we studied biological objects whose eye structure was at different levels of evolutionary development — the elementary light-sensitive formations of worms, the faceted insect eye, the chambered eye of vertebrates and others.

Our investigations produced experimental data which indicate that comparatively small doses of ionizing radiation affect the functional properties of the retina. This is in contrast to the works of certain Soviet [2, 3], American [4], and other authors, who established morphologic and functional changes in the visual apparatus at large doses of ionizing radiation (thousands and tens of thousands of roentgens).

Weak radiation effects (10-50 r) cause definite changes in the functional properties of the retina of isolated frog eyeball, which were determined by the ERG. Irradiation, so to speak, sharply accelerates the process of retinal necrosis [5]. Whole-body irradiation of frogs (doses of 3000 r) lead to an appreciable suppression of the ERG waves (Fig. 1). Simultaneously with the changes in the action currents, we also noted shifts in the value of the rest current of the retina.

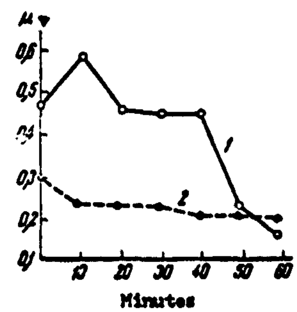

Fig. 1. ERG of the frog eye in the norm and with irradiation. 1) Decrease of the ERG of isolated eyes of intact frogs; 2) decrease of ERG of isolated eyes of frogs irradiated with a dose of 3000 r; time in min is plotted on the abscissa and the ERG in microvolts is on the ordinate.

In another series of experiments the electroretinograms were recorded in unanesthesized rabbits (by means of a contact lens) that were placed in a darkened screened chamber (doses of 500 and 900 r).

An appreciable amplification of the electroretinogram components immediately after irradiation was stopped, was observed in almost all irradiated animals. If the ERG was recorded one hour or more after irradiation, the amplification of the ERG was less expressed. During the following days it underwent considerable fluctuations and dropped prior to the death of the rabbits.

Upon acute irradiation of the animals (rabbits, doses of 500 and 900 r), the changes in the ERG and the adaption of the retina were demonstrated before we noted pathologic shifts in the morphologic picture of the blood and the level of the intraocular pressure. However, in these cases the biocurrents of the eye were retained up to the radiation death of the animals [6].

For a year and a half our laboratory carried out systematic investigations of the chronic effect of ionizing radiation on the functional properties of the retina. In these investigations we used as functional tests: a) the recording of the ERG of awake rabbits by the contact lens-electrode method in response to a light flash and prolonged illumination, b) determination of the rate of the processes of light and dark adaptation of the eye by the ERG method, c) measurement of the intraocular pressure by the Filatov-Kalff method, d) determination of certain formed elements of the blood, e) measurement of the animals' weight.

To determine the limits of normal variations, all these indexes were systematically studied for seven months. Only after this, was the group of rabbits (4 animals) exposed to chronic irradiation with a daily dose of 2.5 r. Other animals (4 animals) acted as the controls.

The investigations of the chronic effect of radiation revealed that when rabbits received a total dose of 125 r, a certain intensification both in the fluctuations of the indexes reflecting the functions of the retina (ERG, light and dark adaptation, intraocular pressure) and the morphological picture of the blood can be noted in the irradiated animals. We detected a certain tendency for the development of leukopenia in the irradiated rabbits, disorders of higher nervous functions, and signs of "radiation cataracts" appeared. All these features, as is known, are characteristic of the first period of chronic radiation sickness. By the end of the investigation (upon increasing the total dose to 400 r) the differences between the irradiated and the control animals increased (Fig. 2). The average value of the electrical response of the retina of irradiated rabbits was 222 microvolts, whereas in the controls it was 388 microvolts.

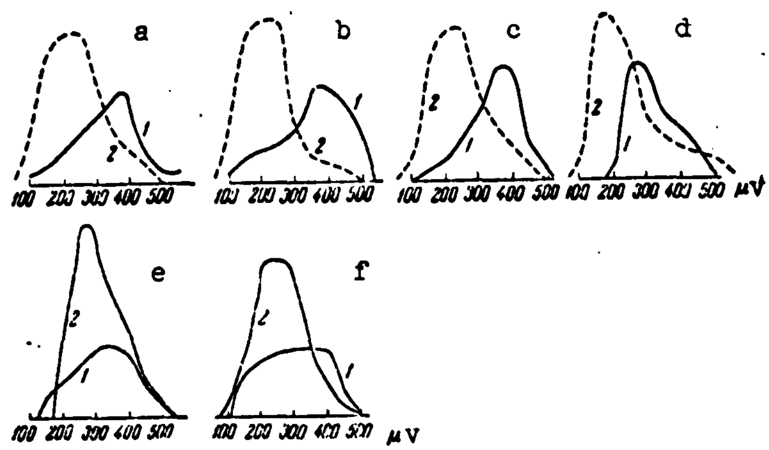

Fig. 2. Distribution of the ERG values detected in the rabbits. On the abscissa is the ERG value in microvolts, on the ordinate is the probability of the demonstration of a response; a, b, c, d: 1) before irradiation (7 months), 2) during chronic irradiation (7 months), e, f: for two unirradiated control rabbits during the same periods.

In the histological investigation of rabbit eyes subjected to chronic irradiation, we noted certain changes from the side of the photoreceptor layer. The fibers of the optic nerve and the ganglion cells of the retina were relatively intact. Noticeable cataractous changes were detected in the lenses.

These facts indicate a tendency for an aggravation of the functional state of the retina of the animals exposed to mild chronic irradiation. This ability of the retina to accumulate and sum the effect of ionizing radiation probably conceals one of the sources of the danger of increasing the natural level of radiation. Since the investigations were performed on a small number of experimental animals, further observations are needed to confirm these deliberations.

We can note substantial differences by comparing the results of our investigations on the effect of ionizing radiation on isolated retina preparations and on the retina in the intact animal. The isolated retina is vastly more subject to the action and damage of radiation than when in an intact animal. In the latter case the radiation changes in the retina are explainable not only by the direct effect of radiation on the tissue, but also by the indirect, reflex effect with the participation of the nervous and endocrine mechanisms, including the compensatory mechanisms.

The high radiosensitivity of the retina was clearly established by us in the experiments on the effect of small amounts of radiophosphorus upon its incorporation into an excised eyeball. However, the experiments on the effect of radioactive strontium on the functional properties of the retina were especially demonstrative (the experiments were performed together with Lebedinskiy [7]). After applying the isotope (dose of 10^{-4} microcuries/cm) to the retina, we observed a rapid attenuation of the bioelectrical activity in all its components. When strontium in a concentration of 10^{-5} microcuries/cm was applied to the preparation, the effect of this action no longer differed from the effect of the control solution of stable strontium.

A calculation showed that under conditions of an active effect of strontium, the radioactivity of the retina was $1.6 \cdot 10^{-10}$ curie, which corresponds to $7 \cdot 10^{-5}$ fermi/min. The natural background of radiostrontium only somewhat exceeded the background radiation. Nevertheless a biological effect was recorded.

As is known, the retina is an exceptionally complex nervous formation which is similar in structure to the cerebral cortex. It contains both photoreceptor elements, rods and cones, and an entire system of synaptic formations, horizontal connections, efferent fibers, and also intrinsic elementary nervous centers, the ganglion cells. Therefore, it is natural that the question arises, what layers and elements of the retina are affected by radiation changes and what is the radiosensitivity of its individual elements.

According to the data of the American investigators Cibris, Noell, and Eichel [4], X-radiation in large doses first destroys the visual receptors, leaving the bipolar and ganglion cells of the retina intact. It is extremely essential that the action of ionizing radiation in this respect is analogous to the effect on the retina of the potent metabolic inhibitor iodoacetate which just as selectively destroys the receptor layer, leaving the ganglion cells untouched.

The relative radioresistance of the retina ganglion cells was confirmed in our laboratory by histochemical investigations. The layer of nerve fibers and the ganglion cells with their processes was revealed with exceptional clarity upon treating plane preparations of the retina by Gomori's method for acid phosphatase. The nerve fibers and ganglion cells with peripherally shifted nuclei are revealed with the same distinctness in the retina of irradiated eyes (dose of 3000 r). Pathological formations resembling a culture flask were frequently noted in the preparations. These formations were nicely "stained," structureless, and have the shape of "worms" with a tail (Fig. 3). Precisely the same formations were detected in the experiments with irradiation of isolated rabbit retina with small doses (35 r). Thus, these formations are associated with irradiation. If we take into account that degeneration of the photoreceptors and the drop of the ERG take place at these doses of irradiation, then retention of the ganglion cells and the conducting pathways does not save the situation since photoreception is impossible anyway.

The faceted eyes of insects, which can perceive high-frequency flickering of light and ultraviolet radiation, are systems that are truely amazing in their complexity and perfection. There are two types of insect eyes. The first is characterized by a relatively simple and slow ERG (for example, the eye of the grasshopper) and cannot perceive a frequency of more than 20-30 oscillations per second. The second type, for example in the blowfly (Calliphora) is characterized by a rapid and highly labile reaction. It can perceive flickering light up to 300 flashes per second.

The object of our investigation was the mallow moth, a destructive pest of the Armenian cotton fields. While studying the problem of the use of ionizing radiation to combat destructive insects, we encountered an interesting form of damage to the visual apparatus upon irradiating these insects.

The mallow moth was X-irradiated in the chrysalis stage with total doses of 1, 5, 10, and 15 curie. The controls were butterflies which had emerged from unirradiated chrysalises. For the electrophysiological investigations of the mallow moth eye, we used the low-frequency electronic oscillograph ENO-1 equipped with a d-c amplifier. A metallic microelectrode (20 microns in diameter) was in contact with a group of ommatidia on the surface of the compound insect eye. In this manner we were the first to record the electroretinogram of the mallow moth which heretofore has not been described in the literature.

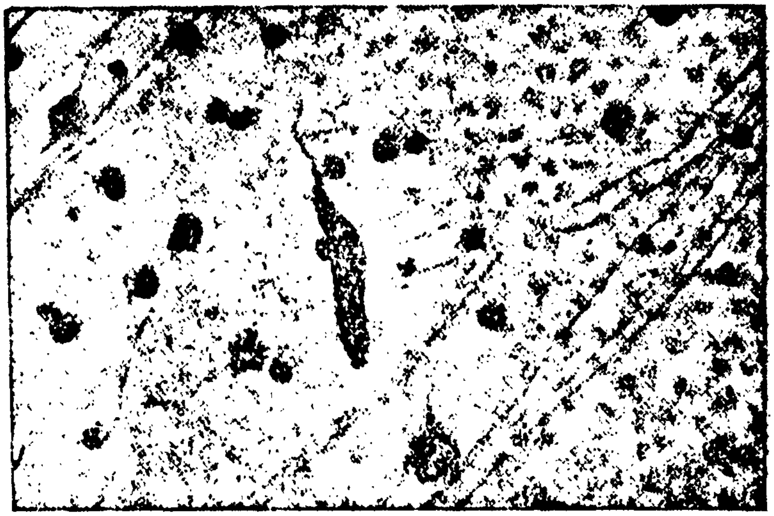

Fig. 3. Total preparation of rabbit retina. Local irradiation of the eye region with a dose of 3000 r (treatment by Gomori method), amplification 6 x 20.

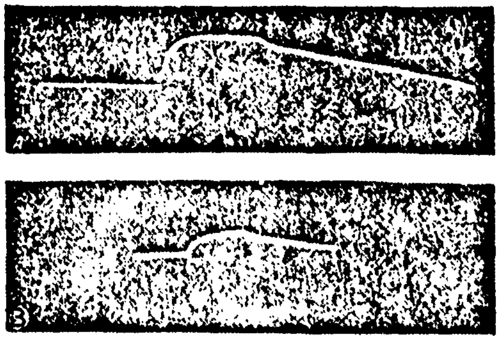

Fig. 4. ERG of eye of normal mallow moth (A) and irradiated insect (B).

The ERG recording of irradiated butterflies revealed substantial changes at doses from 5 curie and higher. In some cases the ERG was absent in general, in others it was recorded in an attenuated form (Fig. 4). It is essential that these disorders in the work of the eye were noted simultaneously with the establishment of an appreciable inhibition of the fertility of the butterflies.

A direct consequence of the exceptionally high radiosensitivity of the retina is its amazing ability to be excited by X-rays and irradiation from radioactive elements, i.e., so-called radiophosphene. This property of the human eye was described by the Russian scientist London [8] and then remained uninvestigated for a long time. The direct effect of radiation on the retinal tissues is of enormous interest for a number of problems under consideration in the present report, since it reveals still unknown mechanisms of photoreceptor excitation in a completely different range of electromagnetic radiations than the visible region of the spectrum. It is essential that the threshold doses of radiation needed for the visual effect be very low (mr).

To estimate the direct or immediate effects of ionizing radiation, we also investigated various photosensitive systems. A photometric analysis of the so-called chromatophore reactions showed that illumination of frog skin led to the concentration of pigment in the chromatophores, which was expressed in a drop of the light transmission coefficient of the skin. Darkness produces the opposite effect. This photosensitive system is insensitive to the effect of ionizing radiation; X-rays in doses from 50 to 5000 r did not evoke any noticeable reactions in the dark.

Another of the reactions we selected, the motor response of the earthworm upon illuminating its surface, proved to be radiosensitive. When we recorded the movements of worms on a recording potentiometer by means of a piezoelectric sensor, we revealed motor reactions of the worm upon irradiation with 200 r, and with better results at a dose of 500 r. We must point out that the motor reaction is not so stable or evident as the light reaction and by no means is demonstrated in each irradiation of the preparation.

Interest in the problem of the direct radiostimulation of tissues has grown considerably on the part of foreign investigators. The studies of Hug [9] revealed a reflex response of certain lower animals to very small doses of irradiation: the movements of the feeler of snails, the shells of mollusks, and of certain insects. The American investigators reported on the direct response of daphnia to irradiation. The observations of roentgenologists have frequently revealed certain reactions following immediately after irradiation: disorders in the normal movements of the intestine and stomach, retardation of the normal passage of food through the stomach, and others. All this characterizes the possibility of the development of "instantaneous" biological and physical responses to the action of ionizing radiation, which can be of substantial importance in over-all radiation damage. Meanwhile this possibility is simply being ignored in most investigations, but by virtue of its fundamental significance it should become the object of special investigations. The retina, it seems to us, is the object which is exceptionally suitable for these investigations.

To explain the results cited in this article, we must take into account the characteristics of the processes which underlie the act of photoperception. It is quite essential that the photochemical phenomena occurring in the retina and determining its sensitivity to light, are intimately dependent on the state of the sulfhydryl groups forming a part of the protein carrier of rhodopsin and other visual pigments. As the investigation of Wald and Brown [10] showed, the bonding of the sulfhydryl groups by thiol poisons precludes the possibility of the normal flow of photochemical and reductive processes and thus should affect the bioelectrical reactions of the retina. Therefore, one of the probable possibilities of explaining the nature of the active effect of ionizing radiation on the functions of the retina lies in the disturbance of the normal state of the sulfhydryl groups owing to their oxidation by the products of water radiolysis. This should inevitably lead to an inhibition of the biochemical cycle in the retina and its electrical response to illumination. The proof of this hypothesis are the data of our laboratory on the direct determination of the content of sulfhydryl groups in homogenates by the amperemetric titration method. In various series of experiments with irradiation of homogenates, isolated retina, and the eye region of whole frogs, we detected a drop in the total content of the sulfhydryl groups at doses as low as 50 r, which progressed with an increase of dose power (Fig. 5). The possibility of direct photochemical changes in photopigments under the effect of ionizing radiation must, apparently, be excluded. This is proved by the investigations of Peskin, who detected bleaching of rhodopsin only at doses in millions of roentgens [11].

We must pay particular attention to the experimentally detected "excitation" phase — an increase in radiosensitivity, an increase in the ERG amplitude during the first phases of the radiation effect. This characteristic of the reactivity of the retina can be compared with the above-mentioned group of experiments on the direct stimulation of the retina by ionizing radiations (X-radiation and radiophosphene). Apparently, in both cases we are dealing with a change of a certain portion of the photosensitive molecules to an excited, metastable state with an increase in the energy supply without ionization. Without a doubt this form of response of the retina differs from a state of damage and indicates the presence in the organism of tissues capable of sustaining the state of direct excitation induced by ionizing radiation. However, this hypothesis requires additional experimental substantiation.

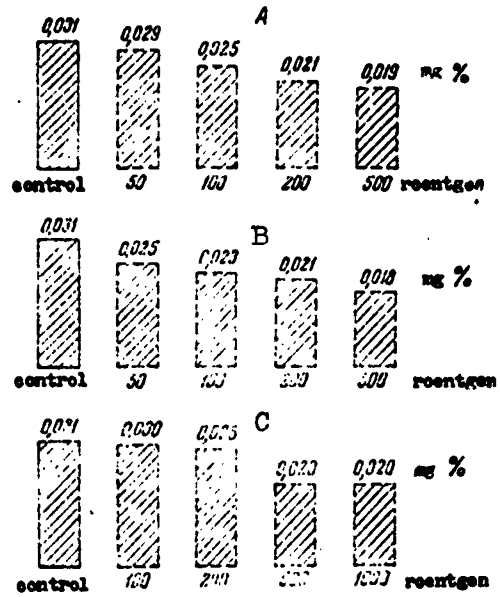

Fig. 5. Content of sulfhydryl groups in homogenates of retina in different forms and doses of irradiation. A) Irradiation of retina homogenates; B) irradiation of isolated retina; C) irradiation of eye region of whole frog.

We note in conclusion that the results of our radiobiological investigations of the retina as a part of the central nervous system agree nicely with the data on the high sensitivity of the CNS itself to the radiation factor, which were obtained by other methods (Livanov [12], Grigor'yev [13], etc.). As a whole these data prove the high radiosensitivity of the nervous system and raise the question with regard to protecting it from the action of ionizing radiation.

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